Family-Based Association Study of Lithium-Related and Other Candidate Genes in Bipolar Disorder

Roy H. Perlis, MD, MSc; Shaun Purcell, PhD; Jesen Fagerness, BS; Andrew Kirby, BS; Tracey L. Petryshen, PhD; Jinbo Fan, PhD; Pamela Sklar, MD, PhD

Context: Association studies in bipolar disorder have been focused on a relatively narrow pool of candidate genes based on a limited understanding of the underlying pathophysiologic features. Recent developments suggest that a broader pool of genes may be associated with this disorder.

Objective: To examine the association between genes related to the lithium mechanism of action, as well as other positional and functional candidates, with bipolar I disorder.

Design: We examined a dense set of haplotype-tagging single-nucleotide polymorphisms using a gene-based test of association.

Participants: Three hundred seventy-nine parent-affected offspring trios.

Results: No genes specifically chosen to probe the action of lithium were associated with bipolar disorder. However, gene-based analysis of sialyltransferase 4A (SIAT4A), tachykinin receptor 1 (TACR1), and γ-aminobutyric acid, β2 receptor subunit (GABRB2) yielded evidence of association (empirical P value, <.005). Among 3 genes associated with schizophrenia or bipolar disorder in multiple previous studies, including dysbindin (DTNBP1), neuregulin (NRG1), and disrupted-in-schizophrenia 1 (DISC1), only DISC1 showed evidence of association in this cohort. In a secondary analysis of these 6 genes among parent-proband trios with a history of psychosis, evidence of the association with SIAT4A was strengthened.

Conclusions: These results suggest novel candidates and 1 gene (DISC1) previously associated with schizophrenia that merit further study in bipolar disorder. However, polymorphisms in major lithium-signaling genes do not appear to contribute substantially to bipolar liability.

Arch Gen Psychiatry. 2008;65(1):53-61
dependent second-messenger signaling.\textsuperscript{20-22} In the second pathway, lithium acts as a selective inhibitor of glycogen synthesis kinase 3β (GSK3β),\textsuperscript{22-25} influencing several downstream pathways including activation of the Wnt-signaling pathway.\textsuperscript{26-30} Perhaps most compellingly, mice that are haploinsufficient for GSK3β display behaviors similar to those of mice receiving long-term treatment with lithium.\textsuperscript{29} The GSK3β pathway has also been postulated to contribute to the observed neuroprotective effects of lithium.\textsuperscript{31} The 2 hypotheses are not mutually exclusive; for example, both pathways appear to converge on the serine/threonine kinase Akt-1 region.\textsuperscript{22} Genes in either of these 2 signaling pathways are therefore candidates for association with the risk for bipolar disorder.

Three other lines of evidence implicate additional candidate genes in bipolar disorder. First, messenger RNA expression studies or similar paradigms have identified additional genes\textsuperscript{32-34} not belonging to 1 of the 2 pathways noted. Some of these are differentially regulated by lithium or by other traditional mood stabilizers, differentially expressed in the brains of patients with bipolar disorder (hereinafter referred to as bipolar patients), or yield proteins that are otherwise implicated in the mechanism of action of mood stabilizers. For example, genes related to oligodendrocyte differentiation or function exhibited differential expression in a postmortem study of bipolar patients,\textsuperscript{33} whereas the traditional mood stabilizer valproate sodium appears to influence histone deacetylation.\textsuperscript{34} Second, a small number of genes known to be expressed in the central nervous system lie under bipolar linkage peaks on 6q and 8q.\textsuperscript{7} Finally, a small number of genes have been shown in multiple studies to be associated with schizophrenia, including disrupted-in-schizophrenia 1 (DISC1),\textsuperscript{35,36} neuregulin (NRG1),\textsuperscript{37,38} and dysbindin (DTNBP1),\textsuperscript{10,44} and an overlap in liability with bipolar disorder has been suggested.\textsuperscript{39} Several other genes have also been associated with the risk of, or the pathways implicated in, schizophrenia or affective illness.\textsuperscript{14,45,46}

Therefore, to identify genes associated with bipolar disorder liability, we conducted a family-based association study examining a select panel of candidate genes based on these hypotheses.

**METHODS**

**SAMPLE DESCRIPTION**

Patient samples were selected from the National Institute of Mental Health [NIMH] Genetics Collaborative Study of Bipolar Disorder waves 1 through 4, details of which have been previously reported.\textsuperscript{47} In brief, that study ascertained subjects in the following order waves 1 through 4, details of which have been previously published.\textsuperscript{54} Interplate concordance was greater than 99.9% and concordance with published Centre d’Étude du Polymorphisme Humain (CEPH) samples, interplate concordance was greater than 99.9%. For the analyzable duplicate samples, interplate concordance was greater than 99.9% and concordance with published Centre d’Étude du Polymorphisme Humain genotypes was 100% (n=1025 genotypes in total).

The single-nucleotide polymorphisms (SNPs) within the candidate genes were selected using a haplotype-tagging (or locus variation–tagging) approach. This approach identifies a set of nonredundant “tag” SNPs that capture common genetic variation in the designated region, allowing for a more efficient screen than typing all SNPs in a region. The tagging approach has been shown to be efficient and powerful for association studies.\textsuperscript{30} In selecting tags, priority was given to known or putative functional SNPs, including exonic SNPs or promoter-region SNPs. First, genetic data for all SNPs in regions encompassing each gene (including 10-kilobase [kb] 5’ and 10-kb 3’ flanking regions) were obtained from the International HapMap Project phase Ic public database (http://www.hapmap.org). The bioinformatics software TAMAL\textsuperscript{41,42} was also used to identify putative functional SNPs in the same gene regions. The SNPs selected from the HapMap and TAMAL databases were submitted to the program Tagger\textsuperscript{43} to identify the subset to be used for tagging; parameters included a minimum coefficient of determination ($r^2$) threshold of 0.8 and minimum minor allele frequency of 0.05. The SNPs selected using TAMAL for their functional importance were forced into the final SNP set, regardless of their tagging performance. For 2 genes previously reported to be associated with schizophrenia, NRG1 and the ionotropic/kainate glutamate receptor 2 (GRK2), the tagging approach was not applied because the large gene size and low linkage disequilibrium would have required a prohibitive number of SNPs to be genotyped; rather, SNPs were selected on the basis of those previously showing evidence of association with schizophrenia.

Genotyping was performed using a single-nucleotide polymorphism platform (Illumina BeadArray at the Center for Genotyping and Analysis of the Broad Institute\textsuperscript{44}). In total, 1536 SNPs were genotyped in 1302 samples; 1 control sample from the Centre d’Étude du Polymorphisme Humain set was also included on each 96-well plate. After data cleaning (eTable 2), the final sample included 1261 autosomal SNPs genotyped in 829 individuals from 225 families and yielded 379 affected-offspring parent-proband trios. Resulting genotype success rates for these SNPs were in excess of 99%, and mean genotyping rates were greater than 99% for all individuals (minimum, 94%). For the analyzable duplicate samples, interplate concordance was greater than 99.9% and concordance with published Centre d’Étude du Polymorphisme Humain genotypes was 100% (n=1025 genotypes in total).

To determine the informativeness of the resulting SNPs for the gene panel, Tagger was rerun with the same parameters but including only the passing SNPs. Although these SNPs were
identified using HapMap phase 1c data, the informativeness for the 1180 HapMap SNPs meeting quality control criteria was estimated using phase II data, released subsequent to our initial genotyping assay development (eTable 1). Of a total of 7762 HapMap phase II SNPs in the tagged genes, 77% were captured with $r^2 \geq 0.8$, with mean $r^2 = 0.83$. For the individual genes, 67 were captured with mean $r^2 = 0.8$ and 108 with mean $r^2 = 0.5$, suggesting that the tag SNPs adequately captured the common variation in these genes.

**ANALYSIS**

As suggested by Neale and Sham, we considered the natural unit of analysis to be the single gene rather than the single SNP or haplotype. We therefore used a gene-based framework to aggregate the single SNP statistics and correct for multiple testing up to the level of the individual gene. Specifically, primary analysis screened for association among the 379 BPI trios using the set-based test implemented in the PLINK association analysis toolset (http://pngu.mgh.harvard.edu/~purcell/plink/) for all 124 genes. This test is similar to that described and shown to be highly efficient by Ott and Hoh: it computes the test statistic ($Q = \sum_{k=1}^{m} T^2_k$) from the single SNP statistics and correct for multiple testing up to the single-gene level. For this analysis, the significance of the SNP combinations including 1 to 5 SNPs were estimated, using 50,000 permutations. Although it would be possible to sum over all SNPs within 1 gene, rather than the best 5, this approach would tend to obscure associations if only 1 or a few SNPs show evidence of association, as we would expect. The transmission disequilibrium test is problematic as a test for association in multiplex families in the presence of linkage because transmissions to affected offspring are not independent. However, the determination of $P$ values by permutation allows transmissions to multiple affected siblings to be analyzed while taking into account this relatedness.

We chose to correct for testing multiple genes for association, within the constraints of available power, by setting a more stringent threshold. We then examined 3-marker haplotypes within the tagged genes, within the constraints of available power, by setting a more stringent threshold. We then examined 3-marker haplotypes within the tagged genes, within the constraints of available power, by setting a more stringent threshold.
sults were essentially unchanged from the primary analysis of the bipolar phenotype (eTable 5). Three-marker sliding-window results for this gene are shown in the Figure (top half of SIAT4A panel [A]).

**COMMENT**

In this large-scale family-based association study of bipolar disorder, we identified evidence of association using a gene-based test for 3 genes from a panel of 124. One gene, SIAT4A, is in a region of chromosome 8 implicated in a meta-analysis of linkage data in bipolar disorder⁷; a second, TACR1, was identified in an expression study of bipolar disorder³²; and a third, GABRB2, was previously implicated in 2 association studies of schizophrenia.⁶¹,⁶² None of the genes related to lithium signaling demonstrated evidence of association.

**Table. Gene-Based Test for Association With Bipolar Disorder**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>No. of SNPs Tested</th>
<th>Mean $r^2$</th>
<th>Proportion of HapMap SNPs Captured With $r^2 &gt; 0.8$</th>
<th>SNP Rank Order$^c$</th>
<th>SNP</th>
<th>Mean $\chi^2$ Test</th>
<th>P Value of Gene$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Replication genes</strong>$^f$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DISC1</td>
<td>chr1:228073965-228483722</td>
<td>54</td>
<td>0.84</td>
<td>0.75</td>
<td>SNP 1 rs11577215 6.62 0.08</td>
<td>SNP 2 rs10864702 6.05 0.04</td>
<td>SNP 3 rs1015101 5.38 0.03</td>
<td>SNP 4 rs1934909 4.86 0.02</td>
</tr>
<tr>
<td>DTNBP1</td>
<td>chr6:19626511-15780272</td>
<td>19</td>
<td>0.91</td>
<td>0.87</td>
<td>SNP 1 rs1000117 3.46 0.38</td>
<td>SNP 2 rs1011313 3.08 0.27</td>
<td>SNP 3 rs1997679 2.72 0.21</td>
<td>SNP 4 rs760666 2.50 0.14</td>
</tr>
<tr>
<td>NRG1</td>
<td>chr8:31618809-32720283</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>SNP 1 rs3249998 3.21 0.41</td>
<td>SNP 2 rs967205 1.97 0.51</td>
<td>SNP 3 rs2466089 1.43 0.55</td>
<td>SNP 4 rs1481743 1.15 0.54</td>
</tr>
</tbody>
</table>

Abbreviations: chr, chromosome; SNP, single-nucleotide polymorphism.

$^a$Mean $r^2$ between genotyped SNPs and all HapMap (International HapMap Project) SNPs in the gene region.

$^b$Proportion of HapMap SNPs in the gene region captured with $r^2 > 0.8$ by the genotyped (tag) SNPs.

$^c$Indicates the number of SNPs (eg, SNP 3 indicates the third most significant SNP in the set).

$^d$Indicates P value is adjusted for all single- and multiple-SNP tests.

$^e$Gene-based test for association with bipolar disorder phenotypes among nonreplication candidate genes with $P < .005$.

$^f$Gene-based test for association with bipolar disorder phenotypes among 3 replication candidate genes.

$^g$Tagging approach was not applied because of gene size.

SIALYTRANSFERASE 4A

Sialytransferase 4A (Online Mendelian Inheritance in Man *607187*), also referred to as ST3 β-galactoside α-2,3-sialyltransferase 1 (ST3GAL1), codes for one of a family of proteins that transfer sialic acid to glycoprotein or glycolipid carbohydrate groups.⁶³ It was included herein because of its location under a bipolar disorder linkage peak on 8q identified in a pooled analysis of linkage data⁷ and prioritized among positional candidates because nerve cell adhesion molecules, key to cell-cell interaction in the developing brain,⁶⁴ are modified by the addition of polysialic acid by sialyltransferases. Changes in the expression of nerve cell adhesion molecule 1 have been noted in the hippocampus in postmortem studies of bipolar patients.⁶⁵ The gene coding for another glycosyltransferase, a mannosyltransferase at 11q23, was shown to be dis-
ruptured by a translocation break point cosegregating with bipolar disorder in one family.66 Finally, a recent report described an association between another sialyltransferase, SIAT8B, and schizophrenia risk.57 Sialyltransferase 4A is known to be expressed in brain and many other tissues68 but is otherwise not well characterized. Our finding that the association is strongest among psychotic patients, particularly in the context of the SIAT8B association, suggests that SIAT4A should be studied among schizophrenia cohorts as well.

**TACHYKININ RECEPTOR 1**

Substance P, alternately referred to as neurokinin and tachykinin, and its primary receptor tachykinin receptor 1 (TACR1) (Online Mendelian Inheritance in Man 600232) have previously been associated with pain and more broadly with stress response, as well as motivation and reward/aversion circuits.69 Studies in mood disorders are limited, but a neuropathology study found differences in the expression pattern of TACR1 among unipolar but not bipolar subjects.70 Substance P antagonists are also known to have antidepressant and anxiolytic effects in animal models,71,72 although human studies remain inconclusive.73

Tachykinin receptor 1 itself was initially reported to be one of a subset of genes regulated by lithium in cultured lymphoblastoid cells.74 After an examination of messenger RNA expression data suggested the gene coding for substance P as a high-priority target in bipolar disorder,32 a small population-based association study failed to find an association in 20 SNPs in 4 genes related to substance P with affective illness, although coverage of TACR1 itself was limited and the cohort was quite small.75 Otherwise, to our knowledge, this gene has not been studied in mood disorder cohorts, and no studies have examined the effects of substance P in bipolar disorder itself.

**GABA<sub>3</sub> β2 RECEPTOR**

The γ-aminobutyric acid<sub>3</sub> (GABA<sub>3</sub>) β2 receptor subunit gene (Online Mendelian Inheritance in Man 600232) is located...
GENES WITH PRIOR REPLICATED EVIDENCE OF ASSOCIATION WITH SCHIZOPHRENIA

The DISC region on 1q42.1 was first identified in a Scottish family in which a chromosome break point translocation segregated with mood and psychotic disorders. Multiple positive linkage studies in schizophrenia or schizoaffective disorder and bipolar disorder followed. The SNPs in the DISC1 region have since been associated with such disorders, and particularly psychosis, in multiple cohorts. Unfortunately, the extent to which the haplotypes examined in these studies overlap has not been fully defined (J Fan, unpublished data, May 23, 2007). As has been noted, studies reported as replication often assess different markers or report different risk haplotypes. Although we did not assess all SNPs included in previous DISC1 publications, of the 37 SNPs showing prior evidence of association across published studies, 20 were directly genotyped or have proxies with $r^2 \geq 0.8$ in our cohort. Only 1 SNP, rs1015101 (associated with schizoaffective disorder in the work of Hodgkinson and colleagues) was nominally associated in our cohort, with $P = .04$. This SNP also tags 1 SNP of a 4-marker haplotype (block 4; rs9432024-rs999710-rs11122359-rs821723) associated with bipolar disorder but not schizophrenia in the same study. Two of the other SNPs in this 4-marker block are tagged with $r^2 \geq 0.8$ and show no evidence of association, while a third is not well-captured with our SNPs. Two additional SNPs in our sample that appear to lie within or adjacent to this block, rs11577215 and rs10864702, also show modest evidence of association (nominal $P = .01$ and $P = .02$, respectively). Thus, although our results cannot be construed as replicating the earlier finding, they are at least consistent with it. Our coverage of other haplotypes associated with bipolar disorder was less complete, but there was no evidence of an association with single SNPs in these regions. Among the SNPs in the region 2 and 3 blocks of Thomson and colleagues, for example, none was associated with $P < .1$ in our study.

In disorders such as diabetes mellitus, targets of drugs known to be effective as treatments have proved to be risk genes for the disorder itself. Notably, then, none of the genes associated with lithium signaling showed significant evidence of association, despite extensive support for the efficacy of lithium in the treatment of bipolar disorder—suggestive evidence that lithium responsiveness may be associated with familial bipolar disorder and isolated positive studies of lithium-related candidate genes. This may simply indicate that the primary genes involved in lithium’s mechanism of action are not those that contribute to liability for bipolar disorder (ie, are dysregulated or dysfunctional in bipolar disorder); instead, lithium may act upstream or downstream of these genes. Alternatively, although our pathway-based approach was as comprehensive as possible based on review of the literature in 2006, other known or unknown genes in these pathways that were not investigated may contribute risk; for example, understanding of GSK3β signaling continues to evolve rapidly. Indeed, a very recent report described association between SNPs in diacylglycerol kinase-eta (DGKH) and bipolar disorder. The diacylglycerol kinases play a role in phosphatidylinositol signaling but were not included in the present study because...
of space constraints. We identified no evidence of association for upstream or downstream genes in that pathway. Finally, although the SNP tagging approach was generally informative for most genes, we cannot exclude the possibility that rarer variation in these genes, or SNPs that were not adequately tagged, are those that confer bipolar risk.

We were unable to detect any significant evidence of association for 2 other genes implicated first in schizophrenia and later as bipolar candidates, NRG1 and DTNBP1, in the gene-based test. In DTNBP1, we directly genotyped or captured by tagging (r² = 1) 8 of the 11 SNPs with evidence of association in schizophrenia, including all of the haplotype-tagging SNPs identified by Mutsuddi et al. In this our results are consistent with negative results from other groups. We also did not detect an association with the set-based test for other genes previously implicated in bipolar disorder, including brain-derived neurotrophic factor (BDNF), the dopamine transporter (SLC6A3), and the serotonin transporter (SLC6A4).

We note 2 primary limitations in this study. First, although it represents one of the larger reported cohorts of bipolar patients, the power to detect moderate effects is still only fair; thus, the possibility of type II error must be considered. Second, all of the reported associations will require replication, because even where the genes implicated overlap with previous reports, the specific SNPs or haplotypes conferring risk apparently do not. Nonetheless, if replicated, these genes may represent novel targets for the development of treatments and diagnostic tools in bipolar disorder.

Submitted for Publication: February 13, 2007; final revision received July 20, 2007; accepted July 24, 2007.

Correspondence: Pamela Sklar, MD, PhD, Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge St, Boston, MA 02114 (sklar@chgr.mgh.harvard.edu).

Financial Disclosure: None reported.

Funding/Support: This study was supported by grants MH062137 (Dr Sklar) and MH067060 (Dr Perlis) from the National Institute of Mental Health and by Independent Investigator (Dr Sklar) and Sidney R Baer Jr Foundation (Dr Sklar) awards from NARSAD.

Additional Information: The eTables and eFigure are available at (www.archgenpsychiatry.com).

REFERENCES

1. Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence, se-
verity, and comorbidity of 12-month DSM-IV disorders in the National Comor-
bidity Survey Replication [published correction appears in Arch Gen Psychia-

2. Mitchell PB, Slade T, Andrews G. Twelve-month prevalence and disability of
DSM-IV bipolar disorder in an Australian general population survey. Psychol

dromal depressive symptoms are associated with functional impairment in pa-
2006;67(10):1551-1560.

4. Hammer M, Gillit M. Stress reactivity in bipolar patients and its relation to


6. Cardno AG, Marshall EA, Coid W, Macdonald AM, Rabe-Hesketh S, Davies NJ, Ven-
turi P, Jones LA, Lewis SW, Shafi MC, Gottesman II, Farmer AE, McDuff P, Revelle AM, Murray RM. Probability estimates for psychosis disorders for the


11. Mendlewicz J, Fieve RR, Stallone F. Relationship between the effectiveness of

12. Goff P, Alda M, Grof E, Zivovs M, Walsh M. Lithium response and genetics of


15. Bergström C, Aström M, Nordqvist-Karlsson B, Adlosssson F, Nylander PO. Relationship between prophylactic effect of lithium therapy and family history of


17. Williams RS, Eames M, Ryves W, Viggers J, Harwood AJ. Loss of a prolyl
oligopeptidase confers resistance to lithium by elevation of inositol (1,4,5) triphosphate. EMBO J. 1999;18(10):2734-2745.

18. Misra PC, Burns HH. “Lithium non-responders” in a lithium clinic. Acta Psy-

19. Misra PC, Burns HH. “Lithium non-responders” in a lithium clinic. Acta Psy-

20. Misra PC, Burns HH. “Lithium non-responders” in a lithium clinic. Acta Psy-

oligopeptidase confers resistance to lithium by elevation of inositol (1,4,5) triphosphate. EMBO J. 1999;18(10):2734-2745.

22. Misra PC, Burns HH. “Lithium non-responders” in a lithium clinic. Acta Psy-

23. Misra PC, Burns HH. “Lithium non-responders” in a lithium clinic. Acta Psy-

24. Misra PC, Burns HH. “Lithium non-responders” in a lithium clinic. Acta Psy-

25. Misra PC, Burns HH. “Lithium non-responders” in a lithium clinic. Acta Psy-

26. Hedgepeth CM, Conrad LJ, Zhang J, Huang HC, Lee VM, Klein PS. Activation of
the Wnt signaling pathway: a molecular mechanism for lithium action. Curr Biol.
2006;49(12):2882-2891.

27. Nelson EW, Gumbiner BM. A cell-free assay system for p-catenin signaling that
recapitulates direct inductive events in the early Xenopus laevis embryo. J Cell

437-442.

29. O'Brien WT, Harper AR, Jové F, Woodgett JR, Maretto S, Piccolo S, Klein PS. Glycogen synthase kinase-3β (GSK-3β) is critical for lithium-induced behavioral and

30. Phiel CJ, Klein PS. Molecular targets of lithium action. Annu Rev Pharmacol
Toxicol. 2001;41:789-813.


